

## ABSTRACT

The present invention relates to a method comprising subjecting a test site of a responsive system to a primary challenge; subjecting the same test site to a secondary challenge, wherein the secondary challenge is designed to enhance and/or prolong a response of the responsive system to the primary challenge, without confounding the response nor altering the mechanism by which the primary challenge elicits a response from the responsive system; and assessing the response subsequent to the secondary challenge. The methods claimed herein also preferably comprise the additional step(s) of subjecting test site(s) to one or more pre-challenge intervention(s) and/or post-challenge intervention(s) and/or concurrent-challenge intervention(s). The methods of the present invention are also useful in a study design, also claimed herein, wherein the study comprises the steps of the method outlined above in addition to comprising the steps of creating one or more controls selected from the group consisting of negative controls, primary controls, secondary controls, positive controls, and mixtures thereof. The use of the novel methods described herein often significantly shorten the duration of and/or number of subjects needed for studies aimed at understanding the etiology of a disease and/or the ability of an intervention or challenge to prevent, cure, heal, and/or reduce and/or induce damage or injury to a responsive system.